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[17] It is not possible, from the data available, to establish unequivocally that the ribosomal activity lost in the presence of anti-A and anti-B serum is present in the precipitate. The possibility cannot be excluded that the component A and B inactivating antibodies become sterically inaccessible and therefore are not precipitated by goat anti-rabbit γ-globulin.

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[22] The precipitation of ribosomal associated component B activity with anti-B serum does not simultaneously precipitate the alkaline phosphatase activity present in such suspensions. This suggests that the messenger RNA molecule that associates with the ribosome is restricted in size.

THE DISAPPEARANCE OF THYMINE DIMERS FROM DNA: AN ERROR-CORRECTING MECHANISM

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Recovery processes associated with ultraviolet irradiation (e.g., photoreactivation, heat reactivation, photoprotection, liquid-holding recovery, host-cell reactivation, and UV reactivation) probably act enzymatically.[1-5] These processes are not additive, but overlap one another,[5-9] indicating that even though they may act in different ways, they operate, at least in part, on identical UV lesions.

Ultraviolet irradiation of DNA results in the formation of intrastrand dimers between adjacent thymine residues.[10] The evidence indicates that such dimers account for a large fraction of the biological effects of UV on DNA.[10, 11] The dimers are split by 330–450 mμ radiation in the presence of an extract from yeast,[12] and one may explain all the biological effects of photoreactivation on transforming DNA in terms of dimer-splitting.[13] Thymine dimers are stable to acid and to enzymic hydrolysis, and may be determined in small numbers when the DNA is labeled with tritium in thymidine. It is thus possible to follow the fate of radiation-induced lesions (thymine dimers produced by UV) in cells that are recovering from the effects of radiation, in the dark as well as in the light.

Thymine dimers block DNA synthesis *in vitro*[14] and *in vivo*.[15] Radiation-resistant cells (defined in terms of colony formation) can recover in the dark from such blocks and resume synthesis; sensitive cells cannot.[15] Cells that are to form colonies must synthesize DNA, and it is reasonable to assume that once DNA synthesis has resumed (and thymine dimers no longer block synthesis), the molecular events associated with the repair of damage to DNA have been completed. Thus, the time for DNA synthesis to resume in UV-irradiated cells can be considered a measure of the recovery time.

During and after the time that recovery takes place in the dark, dimers are conserved inside cells.[15] Therefore, radiation-resistant cells do not recover in the dark by splitting thymine dimers. The following data indicate that in resistant cells the dimers disappear from the acid-insoluble fraction of cells and appear in the acid-soluble fraction. Thus, one step in the molecular repair process is the removal of thymine dimers from the polynucleotide chain.

Methods.—The following five strains of *Escherichia coli* were used: B_{s-1}, a radiation-sensitive strain that shows no host-cell reactivation;[16] B_{s-11}, a radiation-sensitive strain that shows host-cell reactivation;[17] and B, a strain of intermediate sensitivity, forming long filaments after UV, and showing host-cell reactivation. These three strains were obtained from Ruth Hill. Strain B/r (ORNL), obtained from Howard Adler, is a radiation-resistant strain showing host-cell reactivation. Strain 15 T^- has the UV sensitivity of B.

Unless otherwise mentioned in figure legends, bacteria were labeled by growing them at 37°C for 4–5 generation times (35 min/generation) in M9 medium: NH_4Cl, 1 gm; NaCl, 5 gm; Na_2HPO_4, 6 gm; KH_2PO_4, 3 gm; $MgSO_4$, 0.1 gm; glucose, 4 gm; one liter of H_2O, also containing 2 μg/ml of thymidine-methyl-H^3 (6.7 c/mmole), 100 μg/ml of adenosine, and 2.5 mg/ml of casamino acids.[18] After several cycles of centrifugation and resuspension in nonradioactive medium, bacteria were resuspended at a concentration of about 5×10^7/ml in M9 or in M9 without glucose. After UV irradiation, the cells were kept at 37°C (in M9 without glucose) or grown in M9 plus casamino acids. At various times approximately 1 ml of cell suspension was centrifuged. Breakage of cells by sonication at this stage gave results similar to those obtained without sonication. Trichloroacetic acid (TCA)-insoluble and -soluble fractions of cells were obtained as follows. Cells were resuspended in 100 μl H_2O containing 75 μg calf thymus DNA (Worthington), and 100 μl of cold 10% TCA was added. After 5 min the precipitates were spun down and the supernatants were removed. The precipitates were resuspended and precipitated again with TCA. The TCA supernatants were combined, extracted with ether to remove the TCA, and evaporated to dryness. The TCA-insoluble material was washed with 95% ethyl alcohol and dried. Samples were then resuspended in 150 μl of 98% formic acid and hydrolyzed in sealed tubes at 175°C. Chromatographic and counting procedures were the same as used previously.[15] Acid-insoluble samples had 30,000–100,000 (usually 60,000) counts/min.

Monochromatic UV was obtained from a large quartz-prism monochromator. The average intensity through the irradiated samples was approximately 5 ergs/mm^2/sec. Photoreactivating illumination, approximately 10,000 ergs/mm^2/min, between 310 and 400 mμ, was supplied by three black-light lamps placed 15 cm above samples in a 37°C incubator.

Results.—The radiation dose used in most of this work, 200 ergs/mm^2 at 265 mμ, stops DNA synthesis in strains B_{s-1} and B_{s-11},[15, 19] and inactivates colony-forming ability almost completely.[20] It inhibits DNA synthesis for approximately 60 min in strains 15 T^-, B, and B/r,[15, 19, 21] and yields 0.1%, 0.1%, and 10% colony formation, respectively, when cells are plated on M9 agar containing casamino acids.

Two types of experiments, indirect and direct, indicate that the ability of cells to resume DNA synthesis following UV irradiation is associated with a change in the state of the dimers. The indirect experiment makes use of the fact that a

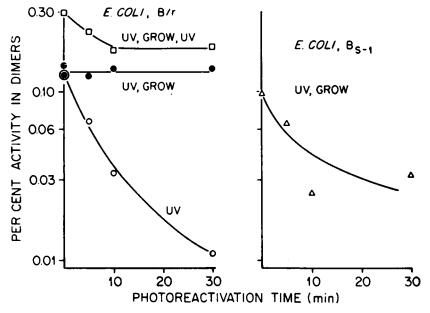

FIG. 1.—The effect of photoreactivating conditions on thymine dimers in bacterial cells. "UV" means a dose of 200 ergs/mm², 265 mμ followed by photoreactivation. "UV, grow" means that the cells were incubated at 37°C in M9 plus casamino acids for 1 hr before exposure to photoreactivating light, and "UV, grow, UV" means that cells were given a second dose of 200 ergs/mm² before photoreactivation.

photoreactivating enzyme preparation from yeast splits dimers in denatured DNA much more slowly than in native DNA and does not split dimers in small oligonucleotides.[22] Figure 1 shows that the dimers in B_{s-1} are split by photoreactivating conditions at 1 hr of growth after UV irradiation. However, in resistant strain B/r only the dimers formed shortly before photoreactivation can be split. The dimers are not split if the cells grow between the initial ultraviolet irradiation and photoreactivating conditions.[23] The implication of these results is that in resistant bacteria the dimers, after a period of growth, are no longer in the native bacterial DNA, even though there has been negligible DNA synthesis during this time.

A direct indication that the dimers in B/r change state while DNA synthesis is blocked is given in Table 1, which shows that the dimers disappear from the acid-insoluble fraction and appear in the soluble fraction of cells growing after UV irradiation. The facts that (*a*) the fraction of dimers in the acid-soluble fraction increases with time, and (*b*) all the dimers appear in this fracton, show that we were observing the removal of dimer-containing oligonucleotides from the bacterial DNA, and not just a general DNA breakdown. Most of the thymine in the acid-soluble fraction comes from the nucleotide pool of the labeled cells.

Figure 2 shows the dimer content of the acid-insoluble fractions of several strains of *E. coli* as a function of time after irradiation. In such experiments both the total radioactive label and the number of dimers in cells are conserved.[24] In the sensitive strain the dimers remain in the acid-insoluble fraction, whereas in resistant strains they disappear from the insoluble and appear in the soluble fraction. The dimers in growing cells[25] are removed more rapidly from the acid-insoluble fraction than those in cells suspended in nonnutrient medium.

The dimers that appear in the acid-soluble fraction of resistant cells seem to be in oligonucleotides because before formic acid hydrolysis the radioactivity associ-

TABLE 1

THE DISTRIBUTION OF THYMINE DIMERS BETWEEN ACID-SOLUBLE AND -INSOLUBLE FRACTIONS OF *E. coli* B/r*

	Time after UV (min)			
	Zero	30	60	90
Counts/min				
Soluble $\widehat{TT}$	0	69	94	80
Soluble T	10,600	7,060	5,490	3,570
Insoluble $\widehat{TT}$	72	26	4	7
Insoluble T	47,700	61,600	62,100	61,200
Total $\widehat{TT}$ / Total T (%)	0.124	0.138	0.145	0.134

* Bacteria irradiated with 200 ergs/mm², 265 mμ were incubated at 37° in M9 plus casamino acids. At various times approximately equal samples were removed, and radioactivity associated with thymine and thymine dimers in the TCA-soluble and -insoluble fractions was determined.

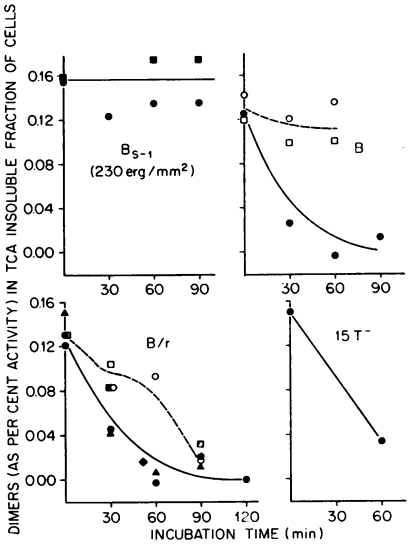

FIG. 2.—The fraction of thymine dimers in the TCA-insoluble fractions of several strains of *E. coli* at various times after irradiation with 200 ergs/mm², 265 mμ (230 ergs/mm² for strain B_{s-1}). Different symbols refer to separate experiments. Closed symbols and solid lines represent cells incubated in nutrient medium (M9 plus casamino acids), and open symbols and dashed lines cells in nonnutrient medium (M9 without glucose).

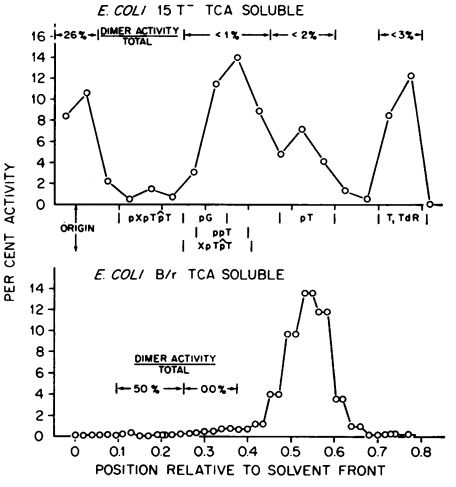

FIG. 3.—Chromatograms of the TCA-soluble fractions of cells incubated for 1 hr in M9 plus casamino acids after 200 ergs/mm², 265 mμ. Labeled cells were grown for approximately two division times in nonradioactive medium before irradiation, thus reducing the sizes of the labeled, acid-soluble pools to 4% for B/r and 2% for 15 T⁻. Acid-soluble material was applied to DEAE-cellulose paper, and the chromatograms were developed with 0.25 *M* NH_4HCO_3. They were cut into strips (1 cm for 15 T⁻, 0.5 cm for B/r), the radioactivity was eluted with 1 *M* NH_4HCO_3, and counted in a scintillation counter. The positions of known markers are indicated. The per cent activity in dimers for several of the regions of the chromatograms are shown.

ated with them migrates on DEAE paper at the same or slower rates than trinucleotides containing dimers[22, 26] (Fig. 3). The actual sizes of the pieces removed from DNA are not given by these data because long polynucleotides (*a*) are not acid-soluble, and (*b*) may be degraded by intracellular nucleases. An estimate of the number of nucleotides removed per dimer may be obtained from the increase in acid-soluble radioactivity in cells during the removal of dimers from DNA.[27] In the experiment on 15 T⁻ shown in Figures 2 and 3 we found that after 60 min growth there were in the acid-soluble fraction from 1 ml of cell suspension increases of 1200 counts/min in thymine and 160 counts/min in dimers. Thus, there were on the average 7.5 thymines or 30 bases hydrolyzed per dimer removed from DNA. The size of the polynucleotides remaining at the origin, shown in Figure 3, cannot be estimated from the value of TT̂/T because this fraction may be contaminated with a slight amount of acid-insoluble material. Presumably the charge on the oligonucleotides prevents them from escaping from cells, and thus accounts for the conservation of dimers in cells.

Discussion.—The disappearance of dimers from the acid-insoluble fraction in growing radiation-resistant cells is accomplished in approximately the time it takes DNA synthesis to resume. Although these data indicate nothing about the

mechanism of dimer removal, nor what, if anything, takes their place in the DNA, it is reasonable to suppose that DNA synthesis resumes in resistant cells because thymine dimers are removed.[28] Since DNA synthesis is necessary for continued cellular proliferation, the removal of dimers from DNA chains may be a necessary first step in the recovery of cells from UV irradiation. However, since in growing B and B/r the dimers disappear from the acid-insoluble fraction at about the same rates, but the ability to form colonies is very different, removal of dimers cannot be the only step leading to recovery.[29] Even in B/r the recovery process by dimer removal is not perfect, because photoreactivation conditions before growth lead to increased colony survival. We may suppose that changes which influence the survival of cells take place before or after removal of dimers. These changes may include a very slow random polymerization around the dimers,[14] further nuclease degradation of the DNA, and the insertion of bases into the vacancies left by the removal of dimer-containing oligonucleotides.[30]

The reactions that are responsible for the removal of dimers from DNA and those that restore DNA to the equivalent of its unirradiated state and the rates of these reactions are sufficient to explain many of the observed recovery phenomena of cells following UV irradiation. In addition, the processes we have observed might be typical of all error-correcting mechanisms involving DNA chains of unnatural or non-Watson-Crick structure.

Summary.—Intrastrand thymine dimers formed by UV irradiation of DNA apparently account for a large fraction of the biological damage to DNA. We have investigated the state of thymine dimers during the time in which resistant strains of *E. coli* recover from the UV-induced delays in DNA synthesis. During this time the dimers disappear from the acid-insoluble fraction of the cells and appear in oligonucleotides in the acid-soluble fraction. Dimers in the acid-soluble phase are not split by photoreactivating conditions. In a sensitive strain the dimers remain in the insoluble phase and remain photoreactivable. Thus, the onset of DNA synthesis is associated with thymine dimer removal, and one step in the recovery of cells from the effects of UV may be the removal of the dimers from DNA. This recovery mechanism could be applicable to other types of damage, or random errors in one strand of a double-stranded DNA.

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[20] The approximate mean lethal doses in ergs/mm^2 at 265 mμ for colony formation are: B_{s-1}, 0.5; B_{s-11}, 1.0; B, 25; B/r, 100. The dose that acts as one effective block to DNA synthesis in strains B_{s-1} and B_{s-11} is 2 ergs/mm^2 (refs. 15, 19).

[21] Hanawalt, P., and R. Setlow, *Biochim. et Biophys. Acta*, **41**, 283 (1960).

[22] Setlow, R. B., W. L. Carrier, and F. J. Bollum, unpublished results.

[23] These results for dimer splitting are similar to those found for reactivation of colony formation. Strains B and B/r are not photoreactivable if incubated for 1 hr in M9 plus casamino acids after UV, whereas B_{s-1} is photoreactivable under these conditions.

[24] In strain B_{s-11} (not as sensitive as B_{s-1} and showing host-cell reactivation) the dimers disappear from the acid-insoluble fraction at about one half the rate of those in B or B/r. However, even for small UV doses, DNA synthesis does not resume, and at the doses used in this work many of the cells lyse (20% by 30 min and 40% by 60 min). It is speculation that in this strain the dimers are "cut out" but that subsequent reactions are unable to "patch" the DNA to its original, native-type configuration, and hence the cells do not recover the ability to make DNA. In B_{s-1} there is a very slow appearance of dimers (20% in 60 min of growth) in the acid-soluble fraction; this may largely be the result of a generalized DNA breakdown in the absence of synthesis rather than a specific reaction that removes dimers from DNA.

[25] Both RNA and protein synthesis continue after UV.

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[27] This is a poor estimate because of uncertainty about the sizes of internal pools and the possible reincorporation of bases after degradation.

[28] Even a small number of blocks in DNA produce a large inhibitory effect on DNA synthesis. Therefore, detectable DNA synthesis does not begin during recovery until almost all the blocks have been removed.

[29] UV irradiation also induces a prolonged division delay in strain B, but not in B/r. The 1/e dose for this delay is about 2 ergs/mm^2 [Deering, R. A., *J. Bacteriol.*, **76**, 123 (1958)]. This value is similar to that necessary to block DNA synthesis in the sensitive strains.

[30] We have been unable to determine if dimers removed from DNA are replaced by normal thymine residues in strain B/r. The small amount of thymidine incorporation observed in irradiated cells placed in labeled medium 15–45 min after irradiation is approximately 10 times that necessary to replace all the dimers. This incorporation may represent the regular synthesis that takes place as blocks to synthesis are removed, a slow synthesis around blocks, or replacement of oligonucleotides removed along with dimers. The material incorporated after 200–400 ergs/mm^2, 265 mμ has the same relative TT frequency as normal DNA because irradiation of it with 30×10^4 ergs/mm^2, 280 mμ produces the same fraction of activity in dimers (0.20–0.22) as found in normal DNA. However, it is different from normal DNA in that it is degraded, in part, to acid-soluble material during irradiation *in vivo* with large UV exposures. Moreover, in irradiated strain 15 T^- incorporated C^{14}-bromouracil cannot be separated from the rest of the DNA by heating and quick cooling, as is possible for unirradiated 15 T^- [Pettijohn, D. E., and P. C. Hanawalt, *Biochim. et Biophys. Acta*, **72**, 127 (1963)].